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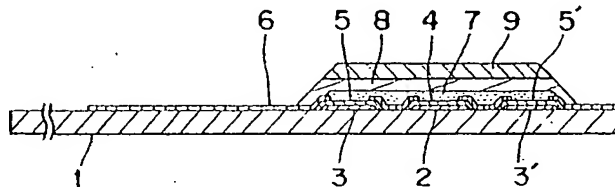
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⑤④ Preparation of biosensor.

57) The present invention relates to a process of preparation of a biosensor comprising forming an electrode system mainly containing carbon on an insulating base plate, treating the surface of electrode system with an organic solvent, and then arranging a reaction layer on the electrode system to give a unified element; by which treatment with the organic solvent the surface of the electrode can be made even and cleaned, and the adhesion of the reaction layer onto the electrode surface can be

improved, and then the exfoliation can be prevented, and the unevenness in each individual sensor can be easily minimized by increasing the evenness of the electrode surface at the production of sensor; and by treatment with the organic solvent an oxide film on the surface of the electrode can be removed so as to give a biosensor having an excellent responsibility and an ability of determination of the substrate concentration with high accuracy.

Fig. 5



EP 0 429 076 A2

make them one body together with a spacer (10) and a cover (11). Introducing a sample liquid onto the enzyme reaction layer through an introducing port (12), the oxidoreductase and the electron acceptor are dissolved into the sample liquid, so that the enzymatic reaction proceeds with a substrate in the sample liquid, and the electron acceptor is reduced. After the completion of the enzymatic reaction the reduced electron acceptor is electrochemically oxidized to determine the concentration of substrate in the sample liquid from the value of the electric current generating in this oxidation.

It was difficult to produce a biosensor having an even quality in the above constitution, because the surface state of the electrode system produced according to a method such as screen printing which is usually adopted to produce a disposable biosensor economically becomes finely varied to give an uneven sensor response. Additionally, according to the above method the wettability of surface of the base plate containing the electrodes is so worse that a solution containing an enzyme is repelled on the electrodes, when coated or spread on the electrodes, so as to hardly form the reaction layer often.

Therefore, as a method of preparing sensors for measuring a specific component in a biological liquid sample such as blood or the like in a simple and rapid way with high accuracy, a method is desired by which an even reaction layer can be easily formed. Further, it is desired that the biosensor has a good storage stability.

SUMMARY OF THE INVENTION

The present invention provides a preparation of a disposable biosensor improved in the evenness among each individual biosensor, responsibility, accuracy and so on by treating the surface of electrodes of the aforementioned conventional disposable biosensor with an organic solvent in the course of the preparation thereof to remove the oxide film or other dirt formed over the electrodes.

BRIEF DESCRIPTION OF THE DRAWINGS

Figs. 1 and 2 are illustrative drawings showing examples of conventional glucose sensors.

Fig. 3 shows a perspective view of a disassembled conventional disposable biosensor.

Fig. 4 is a schematic sectional view of Fig. 3.

Fig. 5 is a schematic sectional view of one embodiment of a biosensor of the present invention.

Fig. 6 is a graph illustrating a response characteristic.

DISCLOSURE OF THE INVENTION

The present invention relates to a process for preparing a biosensor which comprises arranging an electrode system consisting of at least a working electrode and a counter electrode onto an insulating base plate, treating the surface of at least said electrodes for measurement, preferably the whole electrode system with an organic solvent, and then applying a reaction layer onto the treated electrodes.

The surface of the electrodes can be made even and clean by this treatment with an organic solvent, as the result of which the adhesion of the reaction layer onto the surface of the electrode is increased, and the release can be prevented. Further, the arrangement of the reaction layer on the electrode can be made easy, because the wettability of the electrodes is improved thereby, and the formation of bubble can be prevented because the sample liquid can be smoothly introduced onto the electrode. Therefore, a biosensor excellent in a reliability can be obtained according to the present invention.

Furthermore, it becomes easy to minimize unevenness among biosensors by increasing the uniformity of each individual biosensor when it is prepared. Additionally, an oxide film over the electrodes can be easily removed by the treatment with the organic solvent, so that a biosensor can be obtained, by which a substrate concentration can be determined with an excellent responsibility and accuracy.

According to the biosensor of the present invention an adverse influence to the electrodes by solid ingredients such as protein, blood red cell and the like in a blood sample can be prevented by arranging a layer containing a hydrophilic polymer on the electrode. Further, the inactivation of an electrode surface by oxidation can be prevented by the treatment with an organic solvent.

BEST MODES FOR PRACTICING THE INVENTION

EXAMPLE 1

In the following explanatory drawings in the examples, the same numbering is used for common elements and their explanation is in part omitted.

As one embodiment of the biosensor a glucose sensor is illustrated. Fig. 5 is a schematic sectional view of a glucose sensor, which was prepared as one example of biosensor according to the present invention.

Hereafter a process for preparing the sensor is described. Silver paste was printed on an insulating base plate (1) composed of polyethylene terephthalate by means of screen printing to form leads (2), (3), (3'). Next, conductive carbon paste contain-

layer, a PVP layer and a potassium ferricyanide-lecithin layer were formed in a manner similar to the Example 1.

Onto the glucose sensor prepared according to the above process a glucose standard solution 10 μ l was added dropwise as a sample solution, after one minute a pulse voltage of +0.5 V was applied between the electrodes, and then the current after 5 seconds was measured. Similar to the result from the Example 1, a response current corresponding to the glucose concentration was obtained. The good linear relation was also obtained up to the concentration of 900 mg/dl (0.05 mol/l) or more in this Example. In case a whole blood was used as a sample liquid an excellent reproducibility in the response was obtained as in the Example 1.

EXAMPLE 3

According to the same manner as in the Example 1 the several processes for the formation of the insulating layer (6) were repeated, the exposed portions (4) and (5) were polished, and then the obtained substance was subjected to heat treatment at 100 °C for 4 hours in atmosphere. After this treatment the electrode system was exposed over diethyl ether gas circumstances for 60 minutes. Onto the treated electrode system an aqueous solution of CMC (0.5 wt %) containing GOD and potassium ferricyanide is added dropwise and then dried at 40 °C for 10 minutes in a warm air drier to form an enzyme reaction layer to give a glucose sensor.

According to this method, the process for the preparation of the glucose sensor can be simplified by the one time addition of a mixed solution of a hydrophilic polymer, an oxidoreductase and an electron acceptor, and drying it. A temperature for the drying is preferably 20-80 °C in view of maintenance of enzyme activity and short time drying.

Onto the glucose sensor obtained a whole blood 5 μ l was dropped, after one minute a pulse voltage of +0.5 V based on the counter electrode toward the anode was applied on the electrode for measurement, and then the current after 5 seconds was measured. A response current corresponding to the glucose concentration in the whole blood was obtained. Further, when the same sample liquid was dropped thereon, and the voltage was applied after 30 seconds, almost the same response current was observed after one minute. The ground that the above result was obtained was that the reaction layer contained GOD and potassium ferricyanide in mixture, so that the reaction rapidly progressed due to the homogenous solution after the both were dissolved in the sample liquid.

EXAMPLE 4

According to the manner similar to the Example 1, the electrode system was formed on the insulating base plate, and after the polishing and heat treatment the surface of the electrode system was treated with ethyl alcohol. An aqueous solution of CMC (0.5 wt %) as a hydrophilic polymer was spread over the electrodes and dried to form a CMC layer. Onto the CMC layer obtained an aqueous solution of CMC 0.5 wt% containing GOD and potassium ferricyanide was dropped, dried at 40 °C for 10 minutes in a warm air drier to form an enzyme reaction layer to give a glucose sensor.

The enzyme and the electron acceptor could be concentrated upon the working electrode by dropping a solution containing an enzyme on the hydrophilic polymer layer as aforementioned, because the GOD and potassium ferricyanide concentrated upon the dropping point without broadly spreading over the CMC layer due to the rapid absorption of water into the CMC layer, which was a solvent for the GOD, potassium ferricyanide and CMC lately added. As such the GOD and potassium ferricyanide could be concentrated onto the electrode for measurement of the sensor by dropping the mixed aqueous solution containing the GOD after the CMC layer was composed, and it became possible to prepare a sensor by which a stable response was obtained by developing a minimum amount of sample to be needed.

Onto the glucose sensor obtained according to the above process a whole blood 5 μ l was dropped as a sample liquid, and then after one minutes a pulse voltage of + 0.5 V based on the counter electrode toward the anode was applied to the electrode for measurement. When the electric current after 5 seconds was determined, a response current value corresponding to the glucose concentration in the whole blood was obtained. When this test was performed with 30 pieces of the sensor to the same whole blood sample, the variation coefficient was as good as about 3 %.

Though in the above Examples there are illustrated glucose sensors, the present invention should not be construed limitedly to the glucose sensors, but applicable to any system to which an oxidoreductase relates.

As the treatment with an organic solvent there are exemplified the application of ultrasonic wave (frequency: typically 26 kHz) to the electrode as immersed in the organic solvent for several minutes, immersion of the electrode into an organic solvent for about 2 hours and the like, by which a similar effect can be achieved.

The organic solvent usable in the present invention may be selected from any solvents which do not adversely affect, for instance, dissolve, swell

hydrophilic polymer.

9. A process for preparation of a biosensor of the Claim 6, 7 or 8, in which the hydrophilic polymer composing of the reaction layer is selected from the group consisting of cellulose derivatives, gelatine or its derivatives, starch or its derivatives, homo or copolymer containing a residue derived from unsaturated monomer selected from the group consisting of vinyl pyrrolidones, (meth)acrylic acid, salts thereof, maleic anhydride, salts thereof, (meth)acryl amide or salts thereof.

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Fig. 3

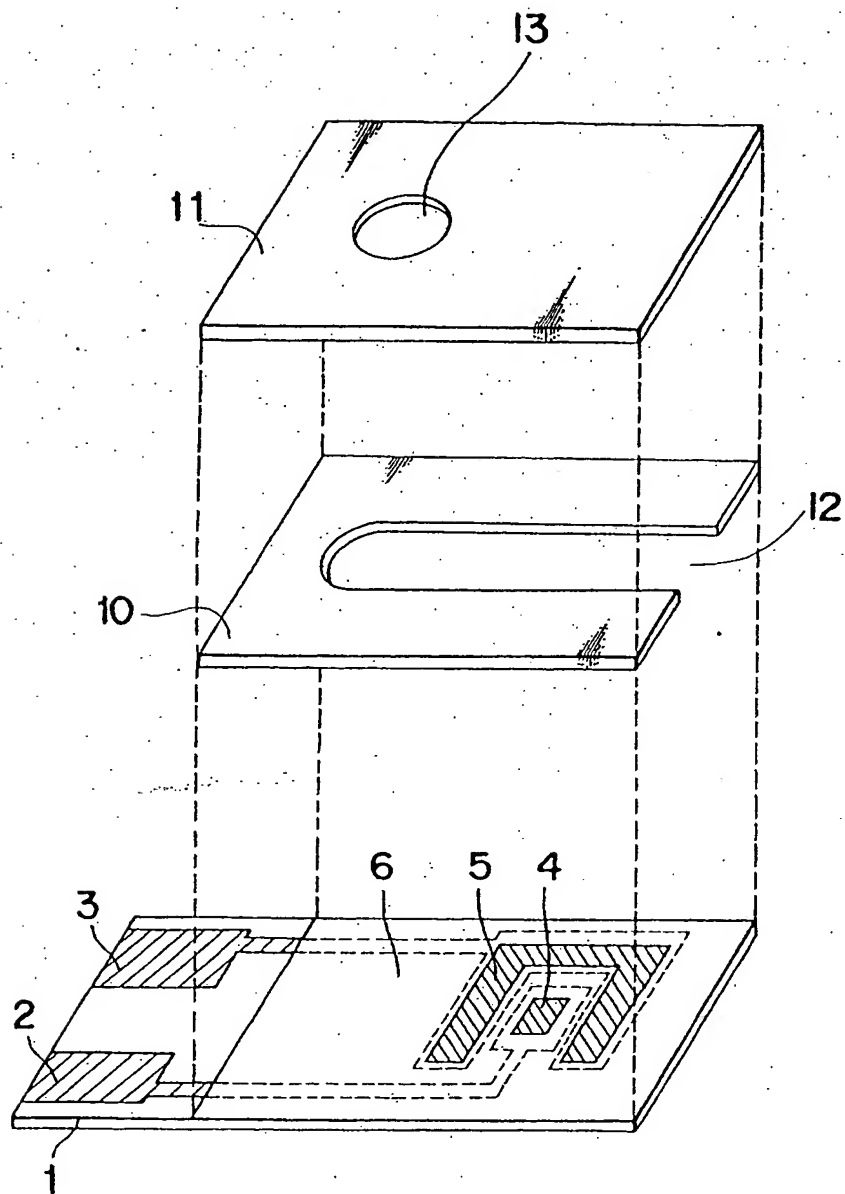


Fig. 6

